Appendix 2. Most Probable Number Determination

JAMES T. PEELER and FOSTER D. MCCLURE

Various methods may be used to estimate the number of bacteria in a particular sample. The 3 methods used most often are the direct microscopic count, the agar plate count, and determination of the most probable number (MPN). Both viable and nonviable organisms are enumerated by the direct microscopic count, whereas only viable organisms are enumerated by the agar plate count and the MPN determination.

The first accurate estimation of the number of viable bacteria was published by McCrady (12). An early use of this technique (4) compared the number of bacteria observed by using solid and liquid media and the estimation of Bacillus coli in water (11). Halvorson and Ziegler (10) published a series of articles on the statistical foundation of the MPN in bacteriology. A general review of the statistical foundations of the MPN is given by Eisenhart and Wilson (9) and Cochran (5). Woodward (16) recommended that MPN tables should be limited. Currently, two American Public Health Association (APHA) methods books (1,2) use abbreviated MPN tables developed by deMan (6-8).

The MPN is an estimate of the density of viable organisms in a sample. To obtain this estimate, the sample must be diluted in such a manner that a more dilute sample will result in fewer positive tubes, which are indicated by the presence of gas or microbial growth. To obtain the MPN, the theory of probability is applied to the test results, with the following assumptions accepted as given. 1) A random distribution of the bacteria exists in the sample. This implies that the likelihood of finding an organism in one part of the sample is the same as that for finding an organism in any other part of the same sample. 2) The bacteria exist as entities, not as groups or clusters, and they do not repel each other. 3) An organism(s) present in the sample will grow in the medium when incubated. 4) The proper conditions exist for growth, i.e., the correct medium has been supplied and the correct incubation temperature is maintained. The number of dilutions to be prepared should be based on the expected population in the sample. The most reliable results are obtained when all tubes at the lower dilution are positive (i.e., microbial growth present) and all tubes at the higher dilution are negative (i.e., microbial growth absent). If a high microbial count is expected, the sample must be diluted to the extent at which the MPN can be obtained (see Chapter 3). Tenfold dilutions are the simplest to perform, and the inoculum is frequently used in 3-, 5-, or 10-tube MPN series; an MPN can be calculated for other than tenfold dilutions. As the number of tubes inoculated for each dilution increases, the confidence limits for the MPN are narrowed. For high-density populations, the MPN is not as precise as a direct plate count and a computed MPN value is an estimate of the population density, not a direct count of living organisms. The MPN is particularly useful for low concentrations of organisms (≤ 10/g) especially in milk, water, and foods where particulate matter may interfere with accurate colony counts. Proper choice of media can give early results for some of the biochemical characteristics, and use of selective media can provide estimates of a mixed population.

A. Tables of MPN and 95% confidence limits

Tables 1-3 present the deMan (6-8) estimates of statistically probable MPN values and the corresponding 95% confidence limits for 3-, 5-, and 10-tube tests. Combinations not in the table have a low probability of occurrence. If the results are not included in the table, the test should be repeated on the original sample. If this is not possible, the MPN can be obtained (3- and 5-tube combinations) from Tables 4 and 5 (3); an equation in section B may also be used to obtain an approximation of MPN combinations. Personal computers may be programmed to compute the MPN and confidence limits (13).

The 95% confidence intervals are interpreted as follows. If the analyst assumes that the true number of organisms lies between the limits, then the assumption will be correct 95% of the time. The tabulated MPN represents a range and not an absolute value.

When more than 3 dilutions of a sample are prepared, the MPN must be determined from only 3 consecutive dilutions (when using Tables 1-3). First, for all dilutions that have all tubes positive, select the highest dilution (smallest sample volume), and then use the next 2 higher dilutions (smaller volumes) (A and B of Tables 6 and 7). When none of the tested dilutions yield all tubes positive, select (if possible) the first 3 consecutive dilutions (sample volumes) for which the middle dilution contains the positive result (C of Tables 6 and 7). Example D illustrates the MPN calculation when a positive result occurs in a higher dilution (smaller sample volume) than the 3 selected. In this case, add the number of positive tubes in the highest dilution, not to exceed 3 or 5. When all tubes are positive, express the MPN as greater than the dilution times the MPN resulting from the combination 3-3-2 or 5-5-4, as appropriate (example E). Read 10-tube values from Table 3 in a similar manner.

Often it is necessary to calculate the MPN from initial sample volumes other than those listed in Tables 1-5. If the largest (greatest) sample volume used for the table reference is 0.01 g, multiply the MPN index listed in the table by 10. Thus, results of a 5-tube MPN determination showing 3 positive 0.01 g portions, 2 positive 0.001 g portions, and 1 positive 0.0001 g portion (3-2-1) are read from Table 2 as 17, and multiplied by 10 to arrive at 170 as the actual MPN/g for the sample. Similarly, if the greatest portion used for the table reference is 1 g rather than 0.1 g, divide the MPN derived from the table by 10. Thus, the result of a 3-tube MPN determination for Salmonella that shows 3 positive 1 g portions, 1 positive 0.1 g portion, and no positive 0.01 g portions (3-1-0) is read from Table 1 as 43 and divided by 10, arriving at 4.3 as the presumptive MPN/g for the sample.

An alternative approach to obtain the MPN/g uses the following formula (14):

(MPN/g from Table) - 100) x dilution factor of middle tube = MPN/g.

For MPN/100 g, multiply by 100.

B. Approximate MPN and 95% confidence limits

Because of the inherent complexity of actually computing MPNs and confidence limits, published tables are often used to determine the MPN. Usually, these tables are restricted to 3, 5, or 10 tubes per dilution. Even with the standard design, irregular data or a laboratory accident can cause the loss of one or more results from a dilution. In the latter case, a dilution series of 5, 4, 4 tubes might yield a positive code of 5-2-0.

Thomas (15) published a simple formula that does not require iteration. MPNs approximated by this formula may not exactly agree with those obtained from theory; however, deviations are usually small. (The approximation may not be acceptable for use in regulatory actions.) The formula is not restricted to the number of tubes and dilutions used and may be applied to all types of tests. The approximation is given by the following equation:

$$\text{MPN/g} = \underline{P}/(\underline{N}\ \underline{T})^{1/2}$$

where $\underline{P}$ is the number of positive tubes, $\underline{N}$ is the total quantity of sample (g) in all negative tubes, and $\underline{T}$ is the total quantity of sample (g) in all tubes.

For example, consider the twofold dilution series given below:

Sample (g)	No. of tubes	No. of positive tubes
8	5	5
4	5	4
2	5	2
1	5	0
0.5	5	1
0.25	5	0

The number of positive tubes is $\underline{P} = (5 + 4 + 2 + 1) = 12$; $\underline{N} = [(8 \times 0) + (4 \times 1) + (2 \times 3) + (1 \times 5) + (0.5 \times 4) + (0.25 \times 5)] = 18.25$; and $\underline{T} = 5\ (8 + 4 + 2 + 1 + 0.5 + 0.25) = 78.75$.

$$\text{MPN/g} = 12/[(18.25)\ (78.75)]^{1/2} = 0.32\text{/g or } 32/100 \text{ g}$$

Estimates of the 95% confidence limits (5) can be obtained from the antilogs base 10 of the following equation.

$$\log\ (\text{MPN/g}) \pm 1.08\ [(\log a)/n)]^{1/2}$$

where $\underline{a}$ is the dilution ratio, and $\underline{n}$ is the number of tubes per dilution. This expression assumes that the dilution ratio is other than 1 to 10 (e.g., 1 to 2). For the 1 to 10 ratio, the ± quantity should be $(1.14)\ (\underline{n})^{1/2}$ for the best estimation. If the number of tubes per dilution ($\underline{n}_i$) is unequal (e.g., lab accident at a dilution) replace $\underline{n}$ in the above expression by the harmonic mean ($\underline{n}_H$) of the number of tubes per dilution ($\underline{n}_i$) for the k dilution. The harmonic mean is defined as $\underline{n}_H = \underline{k}/\ \Sigma(1/\underline{n}_i)^{1}$ and $\underline{k}$ is the number of dilutions. For example, suppose 3 dilutions resulted in $\underline{n}_i$ of 5,4,4. Then $\underline{n}_H = 3/\ [(1/5) + (1/4) + (1/4)]^{1/2} = 3/0.70 = 4.3$.

For the above MPN example with n = 5, the approximate 95% confidence limits are as follows:

$$\log 0.32 \pm (1.08)\ [(\log 2)/5]^{1/2}$$
$$-0.495 \pm 0.265$$

then the lower limit is antilog (-0.76) = 0.17/g or 17/100 g and the upper limit is antilog (-0.23) = 0.59/g or 59/100 g. By comparison, when computed with the deMan tables (7), the MPN would be 0.31/g with confidence limits of 0.16/g and 0.57/g.

References

1. American Public Health Association. 1985. Standard Methods for the Examination of Dairy Products, 15th ed. APHA. Washington, DC.

2. American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater, 17th ed. APHA. Washington, DC.

3. Association of Official Analytical Chemists. 1990. Official Methods of Analysis, 15th ed. AOAC, Arlington, VA.

4. Butterfield, C.T. 1933. Comparison of the enumeration of bacteria by means of solid and liquid media. Public Health Rep. 48:1292-1297.

5. Cochran, W.G. 1950. Estimation of bacterial densities by means of the "Most Probable Number." Biometrics 6:105-116.

6. deMan, J.C. 1975. The probability of most probable numbers. Eur. J. Appl. Microbiol. 1:67-78.

7. deMan, J.C. 1977. MPN tables for more than one test. Eur. J. Appl. Microbiol. 4:307-316.

8. deMan, J.C. 1983. MPN tables, corrected. Eur. J. Appl. Biotechnol. 17:301-305.

9. Eisenhart, C., and P.W. Wilson. 1943. Statistical methods and control in bacteriology. Bacteriol. Rev. 7:57-137.

10. Halvorson, H.O., and N.R. Ziegler. 1933. Application of statistics to problems in bacteriology. J. Bacteriol. 25:101-121; 26:331-339, 26:559-567.

11. Hoskins, J.K. 1933. The most probable number of B. coli in water analysis. J. Am. Water Works Assoc. 25:867-879.

12. McCrady, M.H. 1915. The numerical interpretation of fermentation-tube results. J. Infect. Dis. 17:183-212.

13. Parnow, R.J. 1972. Computer program estimates of bacterial densities by means of the most probable numbers. Food Technol. 26(7):56-62.

14. Silliker, J.H., D.A. Gabis, and A. May. 1979. ICMSF methods studies. XI. Collaborative/comparative studies on determination of coliforms using the most probable number procedure. J. Food Prot. 42:638-644.

15. Thomas, H.A. 1942. Bacterial densities from fermentation tube tests. J. Am. Water Works Assoc. 34:572-576.

16. Woodward, R.L. 1957. How probable is the most probable number? J. Am. Water Works Assoc. 49:1060-1068.

Table 1. Selected MPN and 95% confidence limit (7) estimates for fermentation tube tests with 3 tubes of 0.1, 0.01, and 0.001 g (ml) portions[a]

No. of positive tubes				95% Confidence limits	
0.1	0.01	0.001	MPN/g (ml)[b]	Lower	Upper
0	0	0	<3	-	-
0	1	0	3+	<1	17
1	0	0	4	<1	21
1	0	1	7+	2	27
1	1	0	7	2	28
1	2	0	11+	4	35
2	0	0	9	2	38
2	0	1	14+	5	48
2	1	0	15	5	50
2	1	1	20+	7	60
2	2	0	21	8	62
3	0	0	23	9	130
3	0	1	39	10	180
3	1	0	43	10	210
3	1	1	75	20	280
3	2	0	93	30	380
3	2	1	150	50	500
3	2	2	210+	80	640
3	3	0	240	90	1400
3	3	1	460	100	2400
3	3	2	1100	300	4800
3	3	3	>1100	-	-

[a]Normal results, obtained in 95% of tests, are not followed by a plus (+). Less likely results, obtained in only 4% of tests, are followed by a plus (+). Combinations of positive tubes not shown here occur in less than 1% of tests, and their frequent occurrence indicates that techniques are faulty or that assumptions underlying the MPN estimate are not being fulfilled. MPN estimates for combinations not shown here may be obtained by extrapolation or by Thomas' formulae (15) to the next highest combination shown; for example, a result of 2-0-2 would have an MPN of approximately 20, which is the MPN for a more likely result of 2-1-1.

[b]Multiply all values under MPN/g (ml) by 100 for reporting MPN/100 g (ml).

Table 2. Selected MPN and 95% confidence limits (7) estimates for fermentation tube tests with 5 tubes of 0.1, 0.01, and 0.001 g (ml) portions[a]

No. of positive tubes			MPN/g (ml)[b]	95% Confidence limits	
0.1	0.01	0.001		Lower	Upper
0	0	0	<2	–	–
0	0	1	2+	<1	10
0	1	0	2	<1	10
1	0	0	2	<1	11
1	0	1	4+	1	15
1	1	0	4	1	15
1	2	0	6+	2	18
2	0	0	4	1	17
2	0	1	7+	2	20
2	1	0	7	2	21
2	1	1	9+	3	25
2	2	0	9	3	25
3	0	0	8	3	24
3	0	1	11	4	29
3	1	0	11	4	30
3	1	1	14+	6	35
3	2	0	14	6	35
3	2	1	17+	7	40
3	3	0	17+	7	41
4	0	0	13	5	38
4	0	1	17	7	45
4	1	0	17	7	46
4	1	1	21	9	55
4	2	0	22	9	56
4	2	1	26+	12	65
4	3	0	27	12	67
4	3	1	33+	15	77
4	4	0	34+	16	80
5	0	0	23	9	68
5	0	1	31	13	110
5	1	0	33	14	120
5	1	1	46	20	150
5	1	2	63+	22	180
5	2	0	49	21	170
5	2	1	70	30	210
5	2	2	94+	40	250
5	3	0	79	30	250
5	3	1	110	40	300
5	3	2	140	60	360
5	4	0	130	50	390
5	4	1	170	70	480
5	4	2	220	100	580

Table 2 (continued)

No. of positive tubes			MPN/g (ml)[b]	95% Confidence limits	
0.1	0.01	0.001		Lower	Upper
5	4	3	280+	120	690
5	4	4	350+	160	820
5	5	0	240	100	940
5	5	1	350	100	1300
5	5	2	540	220	2000
5	5	3	920	300	2900
5	5	4	1600	600	5300
5	5	5	>1600	-	-

[a]Normal results, obtained in 95% of tests, are not followed by a plus (+). Less likely results, obtained in only 4% of tests, are followed by a plus (+). Results not shown here occur in less than 1% of tests, and their frequent occurrence indicates that techniques are faulty or that assumptions underlying the MPN estimate are not being fulfilled. MPN estimates for combinations not shown here may be obtained by extrapolation or by Thomas' formulae (15) to the next highest combination that is shown; for example, a result of 4-0-2 would have an MPN of approximately 21, which is the MPN for a more likely result of 4-1-1.

[b]Multiply all figures under MPN/g (ml) by 100 for reporting MPN/100 g (ml).

Table 3. Selected MPN and 95% confidence limits (7) estimates for fermentation tube tests with 10 tubes of 0.1, 0.01, and 0.001 g (ml) portions[a]

No. of positive tubes			MPN/g (ml)[b]	95% Confidence limits	
0.1	0.01	0.001		Lower	Upper
0	0	0	<1	–	–
0	0	1	1+	<1	5
0	1	0	1	<1	5
0	2	0	2+	<1	7
1	0	0	1	<1	5
1	0	1	2+	<1	7
1	1	0	2	<1	7
1	2	0	3+	1	8
2	0	0	2	<1	7
2	0	1	3+	1	9
2	1	0	3	1	9
2	1	1	4+	1	10
2	2	0	4	2	10
2	3	0	5+	2	12
3	0	0	3	1	9
3	0	1	4	2	11
3	1	0	4	2	11
3	1	1	5+	2	13
3	2	0	5	2	13
3	2	1	6+	3	14
3	3	0	6+	3	14
4	0	0	4	2	12
4	0	1	6	2	13
4	1	0	6	2	14
4	1	1	7	3	15
4	2	0	7	3	15
4	2	1	8	4	17
4	3	0	8	4	17
4	4	0	9+	5	19
5	0	0	6	2	15
5	0	1	7	3	16
5	1	0	7	3	17
5	1	1	9	4	18
5	2	0	9	4	18
5	2	1	10+	5	20
5	3	0	10	5	20
5	3	1	11+	6	22
5	4	0	11+	6	22
6	0	0	8	3	18
6	0	1	9	4	20
6	1	0	9	4	20
6	1	1	11	5	22

Table 3 (continued)

No. of positive tubes			MPN/g (ml)[b]	95% Confidence limits	
0.1	0.01	0.001		Lower	Upper
6	2	0	11	5	22
6	2	1	12	6	24
6	3	0	12	6	25
6	3	1	14+	7	27
6	4	0	14+	7	27
6	5	0	15+	8	29
7	0	0	10	5	22
7	0	1	12	6	24
7	0	2	13+	7	27
7	1	0	12	6	25
7	1	1	13	7	27
7	1	2	15+	8	30
7	2	0	13	7	27
7	2	1	15	8	30
7	2	2	17+	9	32
7	3	0	15	8	30
7	3	1	17	9	33
7	4	0	17	9	33
7	4	1	19+	10	36
7	5	0	19+	10	36
8	0	0	13	6	28
8	0	1	15	7	31
8	0	2	17+	8	34
8	1	0	15	7	31
8	1	1	17	9	34
8	1	2	19+	10	37
8	2	0	17	9	35
8	2	1	19	10	38
8	2	2	21+	12	42
8	3	0	19	10	39
8	3	1	21	12	42
8	3	2	24+	13	46
8	4	0	22	12	43
8	4	1	24	13	46
8	5	0	24	13	47
8	5	1	27+	15	51
8	6	0	27+	15	52
9	0	0	17	8	37
9	0	1	19	10	41
9	0	2	22+	11	46
9	1	0	19	10	42
9	1	1	22	11	47
9	1	2	25+	13	52
9	2	0	22	12	47

Table 3 (continued)

No. of positive tubes			MPN/g (ml)[b]	95% Confidence limits	
0.1	0.01	0.001		Lower	Upper
9	2	1	25	13	53
9	2	2	28+	15	58
9	3	0	25	13	54
9	3	1	29	15	60
9	3	2	32+	18	66
9	4	0	29	16	61
9	4	1	33	18	67
9	4	2	37+	20	74
9	5	0	33	18	69
9	5	1	37	20	76
9	5	2	42+	23	83
9	6	0	38	21	77
9	6	1	43+	24	85
9	7	0	44+	24	87
10	0	0	23	12	58
10	0	1	27	14	67
10	0	2	31+	16	77
10	1	0	27	14	69
10	1	1	32	17	79
10	1	2	38	20	92
10	2	0	33	17	83
10	2	1	39	20	96
10	2	2	50	20	110
10	2	3	50+	30	120
10	3	0	40	20	100
10	3	1	50	20	120
10	3	2	60+	30	130
10	3	3	70+	30	150
10	4	0	50	30	120
10	4	1	60	30	140
10	4	2	70	30	160
10	4	3	80+	40	170
10	5	0	60	30	150
10	5	1	70	40	170
10	5	2	90	40	190
10	5	3	100	50	210
10	6	0	80	40	180
10	6	1	90	50	200
10	6	2	110	50	230
10	6	3	120	60	250
10	6	4	140+	70	270
10	7	0	100	50	220
10	7	1	120	60	250
10	7	2	140	70	280

Table 3 (continued)

No. of positive tubes			MPN/g (ml)[b]	95% Confidence limits	
0.1	0.01	0.001		Lower	Upper
10	7	3	150	80	310
10	7	4	170+	90	340
10	8	0	130	60	280
10	8	1	150	80	320
10	8	2	170	90	360
10	8	3	200	100	400
10	8	4	220	120	440
10	8	5	250+	140	480
10	9	0	170	90	380
10	9	1	200	100	430
10	9	2	230	120	490
10	9	3	260	140	560
10	9	4	300	160	640
10	9	5	350	180	720
10	9	6	400+	210	820
10	10	0	240	120	610
10	10	1	290	150	750
10	10	2	350	170	910
10	10	3	400	200	1100
10	10	4	500	300	1400
10	10	5	700	300	1700
10	10	6	900	400	2100
10	10	7	1120	600	2700
10	10	8	1160	800	3700
10	10	9	2300	1100	6000
10	10	10	>2300	-	-

[a]Normal results, obtained in 95% of tests, are not followed by a plus (+). Less likely results, obtained in only 4% of tests, are followed by a plus (+). Combinations of positive tubes not shown here occur in less than 1% of tests, and their frequent occurrence indicates that techniques are faulty or that assumptions underlying the MPN estimate are not being fulfilled. MPN estimates for combinations not shown here may be obtained by extrapolation or by Thomas' formulae (15) to the next highest combination that is shown; for example, a result of 7-0-3 would have an MPN of approximately 15, which is the MPN for a more likely result of 7-2-1.

[b]Multiply all figure under MPN/g (ml) by 100 for reporting MPN/100 g (ml).

Table 4. Most Probable Numbers (MPN) per 1 g of Sample, Using 3 Tubes with Each of 0.1, 0.01, and 0.001 g Portions

Positive Tubes				Positive Tubes				Positive Tubes				Positive Tubes			
0.1	0.01	0.001	MPN	0.1	0.01	0.001	MPN	0.1	0.01	0.001	MPN	0.1	0.01	0.001	MPN
0	0	0	<3	1	0	0	3.6	2	0	0	9.1	3	0	0	23
0	0	1	3	1	0	1	7.2	2	0	1	14	3	0	1	39
0	0	2	6	1	0	2	11	2	0	2	20	3	0	2	64
0	0	3	9	1	0	3	15	2	0	3	26	3	0	3	95
0	1	0	3	1	1	0	7.3	2	1	0	15	3	1	0	43
0	1	1	6.1	1	1	1	11	2	1	1	20	3	1	1	75
0	1	2	9.2	1	1	2	15	2	1	2	27	3	1	2	120
0	1	3	12.	1	1	3	19	2	1	3	34	3	1	3	160
0	2	0	6.2	1	2	0	11	2	2	0	21	3	2	0	93
0	2	1	9.3	1	2	1	15	2	2	1	28	3	2	1	150
0	2	2	12	1	2	2	20	2	2	2	35	3	2	2	210
0	2	3	16	1	2	3	24	2	2	3	42	3	2	3	290
0	3	0	9.4	1	3	0	16	2	3	0	29	3	3	0	240
0	3	1	13	1	3	1	20	2	3	1	36	3	3	1	460
0	3	2	16	1	3	2	24	2	3	2	44	3	3	2	1100
0	3	3	19	1	3	3	29	2	3	3	53	3	3	3	>1100

Table 5. Most Probable Numbers per 100 mL of Sample, Planting 5 Portions in Each of 3 Dilutions in Geometric Series

Number of Positive Tubes				Number of Positive Tubes				Number of Positive Tubes				Number of Positive Tubes				Number of Positive Tubes				Number of Positive Tubes			
10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN
0	0	0		1	0	0.	2.0	2	0	0	4.5	3	0	0	7.8	4	0	0	13	5	0	0	23
0	0	1	1.8	1	0	1	4.0	2	0	1	6.8	3	0	1	11	4	0	1	17	5	0	1	31
0	0	2	3.6	1	0	2	6.0	2	0	2	9.1	3	0	2	13	4	0	2	21	5	0	2	43
0	0	3	5.4	1	0	3	8.0	2	0	3	12	3	0	3	16	4	0	3	25	5	0	3	58
0	0	4	7.2	1	0	4	10	2	0	4	14	3	0	4	20	4	0	4	30	5	0	4	76
0	0	5	9.0	1	0	5	12	2	0	5	16	3	0	5	23	4	0	5	36	5	0	5	95
0	1	0	1.8	1	1	0	4.0	2	1	0	6.8	3	1	0	11	4	1	0	17	5	1	0	33
0	1	1	3.6	1	1	1	6.1	2	1	1	9.2	3	1	1	14	4	1	1	21	5	1	1	46
0	1	2	5.5	1	1	2	8.1	2	1	2	12	3	1	2	17	4	1	2	26	5	1	2	64
0	1	3	7.3	1	1	3	10	2	1	3	14	3	1	3	20	4	1	3	31	5	1	3	84
0	1	4	9.1	1	1	4	12	2	1	4	17	3	1	4	23	4	1	4	36	5	1	4	110
0	1	5	11	1	1	5	14	2	1	5	19	3	1	5	27	4	1	5	42	5	1	5	130
0	2	0	3.7	1	2	0	6.1	2	2	0	9.3	3	2	0	14	4	2	0	22	5	2	0	49
0	2	1	5.5	1	2	1	8.2	2	2	1	12	3	2	1	17	4	2	1	26	5	2	1	70
0	2	2	7.4	1	2	2	10	2	2	2	14	3	2	2	20	4	2	2	32	5	2	2	95
0	2	3	9.2	1	2	3	12	2	2	3	17	3	2	3	24	4	2	3	38	5	2	3	120
0	2	4	11	1	2	4	15	2	2	4	19	3	2	4	27	4	2	4	44	5	2	4	150
0	2	5	13	1	2	5	17	2	2	5	22	3	2	5	31	4	2	5	50	5	2	5	180
0	3	0	5.6	1	3	0	8.3	2	3	0	12	3	3	0	17	4	3	0	27	5	3	0	79
0	3	1	7.4	1	3	1	10	2	3	1	14	3	3	1	21	4	3	1	33	5	3	1	110
0	3	2	9.3	1	3	2	13	2	3	2	17	3	3	2	24	4	3	2	39	5	3	2	140
0	3	3	11	1	3	3	15	2	3	3	20	3	3	3	28	4	3	3	45	5	3	3	180
0	3	4	13	1	3	4	17	2	3	4	22	3	3	4	31	4	3	4	52	5	3	4	210
0	3	5	16	1	3	5	19	2	3	5	25	3	3	5	35	4	3	5	59	5	3	5	250
0	4	0	7.5	1	4	0	11	2	4	0	15	3	4	0	21	4	4	0	34	5	4	0	130
0	4	1	9.4	1	4	1	13	2	4	1	17	3	4	1	24	4	4	1	40	5	4	1	170
0	4	2	11	1	4	2	15	2	4	2	20	3	4	2	28	4	4	2	47	5	4	2	220
0	4	3	13	1	4	3	17	2	4	3	23	3	4	3	32	4	4	3	54	5	4	3	280
0	4	4	15	1	4	4	19	2	4	4	25	3	4	4	36	4	4	4	62	5	4	4	350
0	4	5	17	1	4	5	22	2	4	5	28	3	4	5	40	4	4	5	69	5	4	5	430
0	5	0	9.4	1	5	0	13	2	5	0	17	3	5	0	25	4	5	0	41	5	5	0	240
0	5	1	11	1	5	1	15	2	5	1	20	3	5	1	29	4	5	1	48	5	5	1	350
0	5	2	13	1	5	2	17	2	5	2	23	3	5	2	32	4	5	2	56	5	5	2	540
0	5	3	15	1	5	3	19	2	5	3	26	3	5	3	37	4	5	3	64	5	5	3	920
0	5	4	17	1	5	4	22	2	5	4	29	3	5	4	41	4	5	4	72	5	5	4	1,600
0	5	5	19	1	5	5	24	2	5	5	32	3	5	5	45	4	5	5	81				

Table 6. Examples of determining MPN estimates: 3-tube series, 1 g (ml) sample per tube

Example	Sample amount (g or ml)[a] 0.1	0.01	0.001	0.0001	0.00001	Reported positive values	MPN estimate/g or ml[b]
A	3/3	3/3	2/3	0/3	0/3	3-2-0	930
B	3/3	3/3	3/3	2/3	0/3	3-2-0	9300
C	0/3	0/3	1/3	0/3	0/3	0-1-0	30
D	3/3	3/3	2/3	1/3	1/3	3-2-2	2100
E	3/3	3/3	3/3	3/3	3/3	3-3-3	>110,000

[a]Numerator/denominator = number of positive tubes/number of tubes inoculated.

[b]All figures under MPN/g or ml in this table may be multipled by 100 for reporting MPN/100 g or ml.

Table 7. Examples of determining MPN estimates: 5-tube series, 1 g (ml) sample per tube

Example	Sample amount (g or ml)[a] 0.1	0.01	0.001	0.0001	0.00001	Reported Positive values	MPN estimate/g or ml[b]
A	5/5	5/5	2/5	0/5	0/5	5-2-0	490
B	5/5	5/5	5/5	2/5	0/5	5-2-0	4900
C	0/5	0/5	1/5	0/5	0/5	0-1-0	20
D	5/5	5/5	3/5	1/5	1/5	5-2-2	1400
E	5/5	5/5	5/5	5/5	5/5	5-5-5	>160,000

[a]Numerator/denominator = number of positive tubes/number of tubes inoculated.

[b]All figures under MPN/g or ml in this table may be multipled by 100 for reporting MPN/100 g or ml.